Cardiovascular Pharmacology

Hypercholesterolemia modifies angiotensin II desensitisation and cross talk between α_1-adrenoceptor and angiotensin AT_1 receptor in rabbit aorta

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ABSTRACT

This study characterised the effect of a hypercholesterolemic diet on the interactions of hormone receptors in the rabbit aorta, both in homologous desensitisation to angiotensin II and cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors. Rabbits were fed either a normal chow or a diet containing 1% cholesterol for 6–7-weeks. Isometric contractions were measured in endothelium-intact or endothelium-removed aortic rings from control and hypercholesterolemic rabbits. Concentration response curves to angiotensin II or noradrenaline incubated with or without prazosin or losartan were performed. In another group, the resting potential was recorded at baseline and following angiotensin II or noradrenaline stimulation. Rabbits fed a hypercholesterolemic diet showed higher plasma levels of total cholesterol and LDL-cholesterol and impaired relaxation to acetylcholine. Homologous desensitisation to angiotensin II was found in endothelium-intact but not in endothelium-removed arteries. Cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors was modified with respect to physiological conditions. In control rabbits, angiotensin II desensitised the noradrenaline response but noradrenaline did not modify the angiotensin II-response. However, in hypercholesterolemic rabbits, angiotensin II sensitised the noradrenaline-response and noradrenaline desensitised the angiotensin II-response. Furthermore, the resting potential remains hyperpolarised after noradrenaline stimulation in hypercholesterolemic rabbits. Modifications in homologous desensitisation to angiotensin II and cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors suggest that hypercholesterolemia induces early tissue dysfunction by altering endothelial and smooth muscle cell regulatory properties. This may be one of the mechanisms by which hypercholesterolemia could be involved in the onset and progression of chronic vascular diseases such as hypertension and arteriosclerosis.

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1. Introduction

Functional abnormalities of the vascular endothelium are recognised as significant contributors involved in the genesis or perpetuation of pathological states such as hypertension. The observation that lipid accumulation in the blood vessel walls during hypercholesterolemia produces endothelium dysfunction even before morphological changes occur (Sorensen et al., 1994) may be an additional mechanism to link hypercholesterolemia and hypertension. In a previous work, Jerez et al. (2008) reported endothelial dysfunction and increases in the angiotensin II response in aortic rings from hypercholesterolemic rabbits.

However, the exposure to elevated catecholamines or angiotensin II results in homologous desensitisation of both adrenergic and angiotensin AT_1 receptor-mediated vascular smooth muscle contraction in both rat and rabbit aorta (Silva et al., 1988; Kuttan and Sim, 1993). Previous studies also reported endothelium dependent and independent desensitisation to angiotensin II in rabbit aorta (Gruetter et al., 1987; Jerez et al., 2001), although the role of endothelial dysfunction induced by hypercholesterolemia on the desensitisation to angiotensin II remains to be elucidated.

Receptor cross talk is paramount to normal cellular function. Activation of one receptor by its ligand often affects cellular responses to other hormones and neurotransmitters. It has been shown that functional receptor hetero-oligomers could be formed between many potential pairs of G protein-coupled receptor family members when they are expressed in the same cell (Franco et al., 2008). Moreover, desensitisation of cellular responses to a given neurotransmitter could affect cellular responses to other neurotransmitter(s) that have receptors expressed in the same cell type and form functional hetero-oligomers with receptors of the given neurotransmitter (Gurwitz, 2000).

Cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors has previously been demonstrated. Vascular AT_1 gene expression and

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receptor protein are regulated by ambient noradrenaline levels, and noradrenaline induced downregulation of AT_1 mRNA and receptor protein (Du et al., 1997). Moreover, angiotensin II enhances transcription and expression of α_1-adrenoceptors in vascular smooth muscle cells (Hu et al., 1995). However, Li et al. (1997) found that angiotensin II selectively downregulates α_{1a}-adrenoceptors subtype mRNA and its corresponding receptors in neonatal rat cardiac myocytes. In addition to these data, Jerez et al. (2004) demonstrated an endothelium-dependent cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors in the smooth muscle of rabbit aorta. Reciprocal regulation between the renin–angiotensin system and the sympathetic nervous system may play an important role in the control of blood pressure homeostasis. By extension, it seems reasonable to assume that lack of negative feedback on angiotensin AT_1 receptor by angiotensin II and/or noradrenaline may exist in pathophysiological conditions (Yang et al., 1996; Crespo, 2000).

Based on these findings, the aim of this paper is to study the effect of a rich cholesterol diet in the interaction agonist-receptor using like a model of this interaction the phenomena of homologous desensitisation to angiotensin II and cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors. This study explores for the first time why the functional mechanisms of early hypercholesterolemia may modify the smooth muscle response to vasoactive agonists responsible for maintaining the homeostasis of blood pressure.

2. Materials and methods

2.1. Animals

Male hybrid-Flanders rabbits from a slaughterhouse initially weighing 850–1000 g were used in this study. The animals were maintained under controlled light and temperature conditions and fed either normal rabbit chow or a diet containing 1% cholesterol for 6–7 weeks with free access to tap water. At the end of the 6–7-week dietary intervention, food was withdrawn for 12 h, and the rabbits were weighed and then anesthetised with ketamine (75 mg/kg). All experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No 85–23, revised 1996) and approved by our Institutional Committee for the ethical care of Animals.

2.2. Blood pressure measurement

Arterial blood pressure was directly measured in the carotid artery through a catheter connected to a pressure transducer (Gould, USA) and recorded on a data acquisition system (BIOPAC MP100). After arterial pressure measurement, blood samples were collected in prechilled glass tubes containing EDTA 10^{-7} M through the catheter inserted in the carotid artery. Plasma cholesterol, high-density lipoproteins (HDL)-cholesterol, low-density lipoproteins (LDL)-cholesterol, triglycerides and glucose were measured by a colorimetric reaction with commercial kits (Wiener, Argentina).

2.3. Isometric tension measurement

Thoracic aortic rings (5 mm) were mounted in a 10-ml organ bath containing Krebs solution of the following composition (mM): NaCl 128, KCl 4.7, $NaHCO_3$ 14.4, NaH_2PO_4 1.2, Na_2-EDTA 0.1, $CaCl_2$ 2.5, glucose 11.1, pH 7.2. Krebs solution was kept at 37 °C and aerated with 95% O_2 and 5% CO_2. Isometric contractions were measured with force-displacement transducers and recorded under an initial tension of 2 g, which was found to be the optimal tension for KCl-induced contraction (100 mM). The endothelium was kept intact in some rings (endothelium-intact arteries) and was removed by rubbing the luminal surface (endothelium-removed arteries) in other groups.

To assess endothelial function, aortic rings were contracted with noradrenaline 5.10^{-6} M and concentration response curves to acetylcholine (10^{-8} to 10^{-5} M) were calculated.

To analyse homologous desensitisation to angiotensin II, endothelium-intact or endothelium-removed aortic rings from control and hypercholesterolemic rabbits were exposed to increasing doses of angiotensin II (10^{-10} to 10^{-6} M) to construct the initial concentration response curve. Rings were washed with Krebs solution for 90 min and a second concentration response curve was calculated.

To evaluate the cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors, endothelium-intact arteries from hypercholesterolemic and control rabbits were treated with either one concentration response curve to noradrenaline (10^{-8} to 10^{-3} M) or one concentration response curve to angiotensin II. Rings were rinsed and a 30 min recovery period was allowed prior to performing the following: a) a subsequent concentration response curve to angiotensin II in the rings previously treated with noradrenaline, or b) a subsequent concentration response curve to noradrenaline in arteries pretreated with angiotensin II. To evaluate the role of α_1-adrenoceptors, a specific antagonist (prazosin 10^{-6} M) was added to the bath 30 min before the concentration response curve to angiotensin II. To study the role of angiotensin AT_1 receptors in the cross talk, a specific antagonist (losartan 10^{-7} M) was added to the bath 30 min before the concentration response curve to noradrenaline or angiotensin II. Both of the antagonists used were removed from the bath immediately after the agonist stimulation.

Results are expressed as mg of isometric contraction.

2.4. Electrophysiological studies

Aortic arteries were removed from rabbits as described above and were cut open along the long axis before being pinned, intimal surface upwards, to the silicone rubber base of an organ chamber (volume 5 ml) with a temperature controller (37 °C) and aerated with 95% O_2 and 5% CO_2. Segments were immersed in Krebs solution. Smooth muscle cell impalement was performed from the intimal side of the vessels. The transmembrane potential was recorded with glass electrodes filled with 3 M KCl (tip resistance 50–80 Ω), which were connected to the headstage of a recording amplifier equipped with capacitance neutralisation. The electrophysiological signal was continuously monitored on an oscilloscope and simultaneously recorded on paper. Successful impalements were signalled by a sudden negative drop in potential from the baseline (zero potential reference) followed by a stable negative potential for at least 10 min and were held under current clamp conditions.

In endothelium-intact arteries, the resting membrane potentials (P_m) were measured at baseline conditions (B_0) as well as exposed to angiotensin II 10^{-6} M or noradrenaline 10^{-6} M stimulation (1 min: A_0 or NA_0; 10 min: A_{10} or NA_{10}). Arteries were washed with Krebs solution and the P_m was re-measured to ensure recovery (20 min after washing: W_{20}). Depolarising KCl solution was finally added.

Values are expressed in mV as differences: $(B) = B_0$ minus A_0 or NA_0, and (Angiotensin II or NA) $= A_0$ or NA_0 minus A_{10} or NA_{10}, $(W_{20}) = W_{20}$ minus B_0.

2.5. Data analysis

Data are presented as the mean ± S.E.M and were analysed by ANOVA with replications and Duncan's test to evaluate concentration response curve. The pEC_{50} (negative log of molar agonist concentration inducing 50% of the maximal contraction) and the maximal contractile response were calculated using a curve-fitting analysis program. A Student's t test (paired or unpaired) was used to compare pEC_{50} values or maximal response. $P < 0.05$ was considered statistically significant (two-tail test).

3. Results

Rabbits fed a diet enriched with cholesterol showed higher plasma levels of total cholesterol and LDL-cholesterol than animals fed a control diet. However, HDL-cholesterol, triglycerides and glucose plasma levels were similar in both groups. At the end of the experiment, no differences were found in body weight or blood pressure between the diet groups (Table 1).

3.1. Response to acetylcholine

Acetylcholine caused endothelium-dependent relaxation in a concentration-dependent manner in both diet groups. However, endothelium-dependent relaxation to acetylcholine decreased significantly in aorta from rabbits fed a cholesterol-enriched diet (Fig. 1). The maximum relaxation was induced by acetylcholine (10^{-5} M).

3.2. Homologous desensitisation to angiotensin II in aortic rings from control and hypercholesterolemic rabbits

The contractile response to angiotensin II was dose-dependent. In aortic rings from control rabbits, the pEC_{50} values of the initial concentration response curve were similar in arteries with endothelium-intact and endothelium-removed (8.18 ± 0.12 vs. 8.30 ± 0.12, respectively; $n = 10$). A significant shift to the right following the second exposure to angiotensin II was observed in both groups (Fig. 2). The rightward shift was similar and no significant differences were found in maximal response between endothelium-intact and endothelium-removed in both the first and second concentration response curves (ANOVA and Duncan's test, $n = 10$).

Angiotensin II-contractile response was increased in endothelium-intact arteries from rabbits fed a hypercholesterolemic diet compared with rabbits fed a control diet (3959 ± 667 mg, hypercholesterolemic vs. 3162 ± 671 mg, control; $n = 10$, $P < 0.05$). Similar results were obtained with endothelium-removed arteries (4076 ± 842 mg, hypercholesterolemic vs. 3152 ± 511 mg, control; $n = 10$, $P < 0.05$).

In aortic rings from hypercholesterolemic rabbits, the pEC_{50} values of the first concentration response curve were similar in arteries endothelium-intact and endothelium-removed (8.05 ± 0.06 vs. 8.09 ± 0.07, respectively; $n = 10$). A significant shift to the right following the second exposure to angiotensin II was observed in endothelium-intact but not in endothelium-removed arteries (Fig. 2). In addition, the maximal response of a second concentration response curve was enhanced in endothelium-removed preparations (Fig. 2).

3.3. Cross talk: effect of prazosin and losartan treatment on contractile response to angiotensin II

Noradrenaline treatment prior to the exposure to angiotensin II did not modify the maximal response or pEC_{50} in aortic rings from
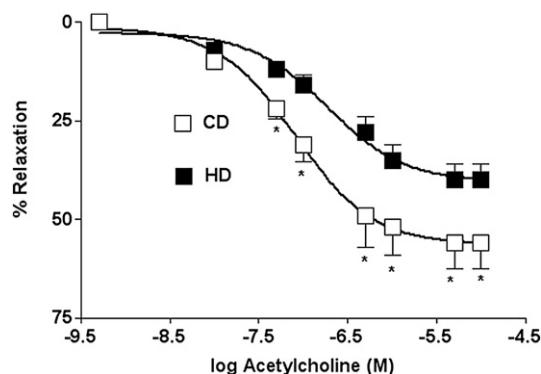

Fig. 1. Vasorelaxation effect induced by acetylcholine (10^{-8}–10^{-5} M) in rabbit aortic rings precontracted with a submaximal dose of noradrenaline (5×10^{-6} M) from rabbits fed a hypercholesterolemic (HD) or a control diet (CD). Each data point represents the mean of 10 experiments and vertical lines indicate the S.E.M. *$P < 0.05$ indicates statistically significant differences between hypercholesterolemic and control diets.

control rabbits but did induce desensitisation in aortic rings from hypercholesterolemic rabbits (Table 2).

In the presence of prazosin, a significant shift to the right was observed in the concentration response curve to angiotensin II in aortic rings from control ($pEC_{50} = 8.25 \pm 0.06$, control; $pEC_{50} = 7.74 \pm 0.15$ prazosin, $P < 0.01$, $n = 10$) and hypercholesterolemic rabbits ($pEC_{50} = 7.98 \pm 0.05$, control; $pEC_{50} = 7.68 \pm 0.04$ prazosin, $P < 0.01$, $n = 10$). The maximal contractile response was increased in control (3059 ± 245 mg, control, vs. 4456 ± 556 mg, prazosin; $P < 0.05$, $n = 10$) but was not modified in hypercholesterolemic rabbits (4288 ± 452 mg, control, vs. 3737 ± 688 mg, prazosin; n.s, $n = 10$). Furthermore, prazosin treatment enhanced the desensitisation to angiotensin II induced by noradrenaline stimulation in hypercholesterolemic arteries ($pEC_{50} = 7.63 \pm 0.07$, arteries treated with prazosin; $pEC_{50} = 7.34 \pm 0.07$, arteries treated with noradrenaline in presence of prazosin before angiotensin II stimulation, $P < 0.05$, $n = 10$).

Losartan treatment induced a shift to the right in the concentration response curve to angiotensin II both in control and hypercholesterolemic rabbits (Table 2). In addition, incubation with losartan during the concentration response curve to noradrenaline performed before angiotensin II-stimulation increased the desensitisation induced by noradrenaline in hypercholesterolemic but not in control arteries (Table 2). Insufficient removal of losartan before the concentration response curve to angiotensin II does not account for this effect, as a significant difference was observed between arteries incubated with losartan ($pEC_{50} = 7.11 \pm 0.07$) and arteries previously treated with noradrenaline plus losartan ($pEC_{50} = 6.43 \pm 0.3$).

3.4. Cross talk: effect of losartan treatment on contractile response to noradrenaline

Contractile response to noradrenaline was similar in control and hypercholesterolemic rabbits (Table 3). One concentration response curve to angiotensin II induced a shift to the right of the subsequent concentration response curve to noradrenaline in control arteries. In contrast, angiotensin II pretreatment induced a shift to the left in the concentration response curve to noradrenaline in hypercholesterolemic rabbit aortic rings (Table 3).

In control arteries, losartan did not modify the response to noradrenaline. However, in hypercholesterolemic arteries, losartan induced a shift to the right in the concentration response curve to noradrenaline (Table 3). Incubation of hypercholesterolemic arteries with losartan during the concentration response curve to angiotensin II not only blocked the sensitisation of noradrenaline-response induced by

Table 1
Lipids and blood glucose levels, arterial blood pressure and weight values from rabbits fed on a control diet or a diet containing 1% cholesterol.

	Control diet	Hypercholesterolemic diet
Cholesterol total	0.55 ± 0.11 g/l	6.42 ± 1.05 g/l [a]
LDL-cholesterol	0.22 ± 0.09 g/l	4.03 ± 0.80 g/l [a]
HDL-cholesterol	0.28 ± 0.11 g/l	0.75 ± 0.22 g/l
Triglycerides	1.74 ± 0.38 g/l	1.60 ± 0.38 g/l
Blood pressure	81 ± 10 mm Hg	88 ± 8 mm Hg
Blood glucose	105 ± 13 mg/dl	98 ± 4 mg/dl
Weight	2072 ± 0.178 g	2198 ± 0.115 g

Data are expressed as means $\pm$ S.E.M. of 16 rabbits. LDL: low density lipoproteins. HDL: high density lipoproteins.

[a] $P < 0.05$ indicates statistically significant differences between rabbits fed on a control diet and rabbits fed on a diet enriched with cholesterol (paired *t* test).
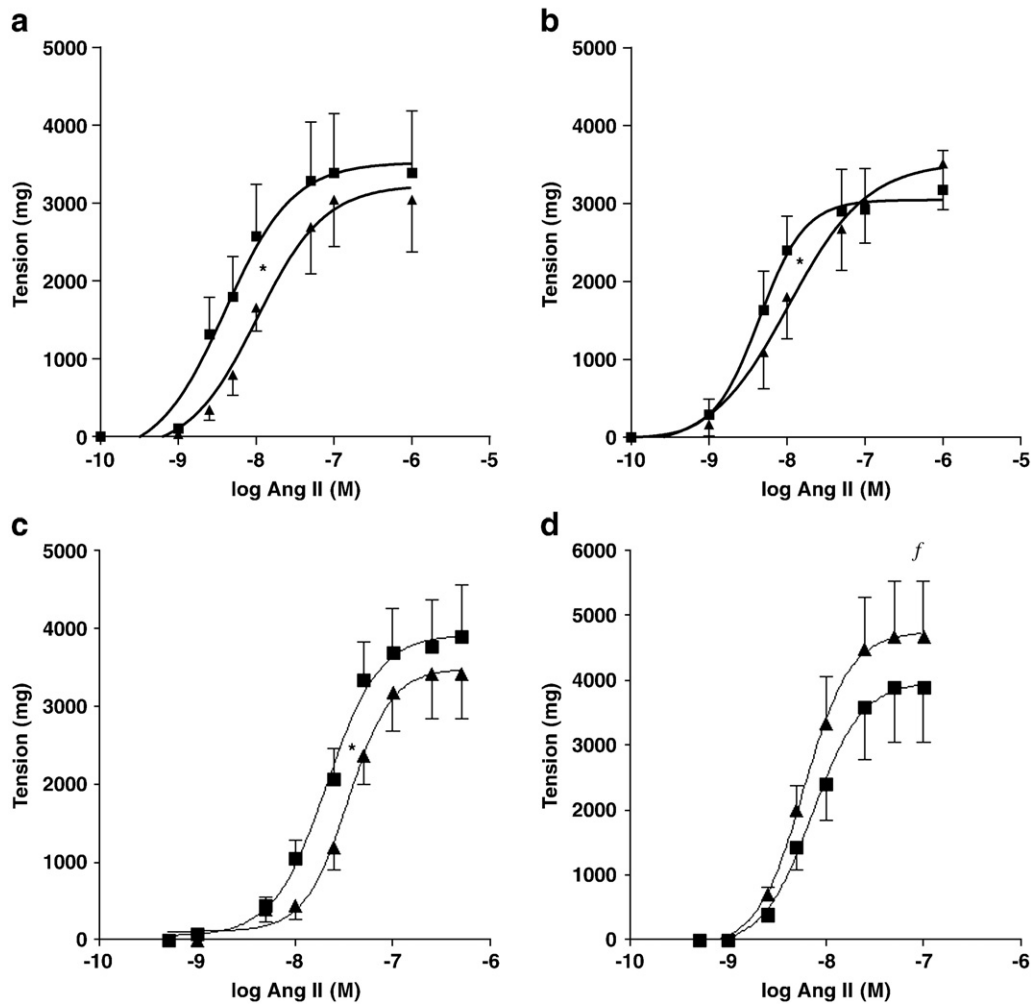
Fig. 2. Concentration response curves to angiotensin II (-■-: first; -▲-: second) in aortic rings with intact endothelium (*a* and *c*) or endothelium removed (*b* and *d*) from rabbits fed a control (*a* and *b*) or a hypercholesterolemic diet (*c* and *d*). Each data point represents the mean of 10 experiments and vertical lines indicate the S.E.M. *$P < 0.05$ indicates statistically significant differences in pEC$_{50}$ between the first and second concentration response curve. *f* $P < 0.05$ indicates statistically significant differences in maximal response between the first and second concentration response curve.

angiotensin II stimulation but was also able to induce desensitisation. No effect on the maximal response was observed in these aortic rings (Table 3).

3.5. Resting membrane potentials measurement

No differences were found between hypercholesterolemic and control rabbits arteries at baseline conditions (*B*). The P_m of arteries from rabbits fed a hypercholesterolemic diet was significantly less

Table 2
Maximal response and pEC$_{50}$ to angiotensin II in endothelium-intact aorta from control (CD) and hypercholesterolemic (HD) rabbits.

	E_{max} (mg)	pEC$_{50}$ (logM)	E_{max} (mg)	pEC$_{50}$ (logM)
	CD		HD	
Ang II	3059 ± 245	8.17 ± 0.07	4288 ± 452^a	7.98 ± 0.05
Ang II/NA	3516 ± 550	8.17 ± 0.05	4027 ± 718	$7.63 \pm 0.07^{a,b}$
Ang II + Los	2597 ± 618	7.15 ± 0.06^b	3791 ± 592^a	7.11 ± 0.07^b
Ang II/NA + Los	2047 ± 673	7.44 ± 0.09^b	4555 ± 1003^a	$6.45 \pm 0.3^{a,b,c}$

E_{max}: maximal response; Ang II: angiotensin II; Ang II/NA: arteries treated with one concentration response curve to noradrenaline (NA) before angiotensin II stimulation; Ang II + Los: arteries incubated with losartan (Los); Ang II/NA + Los: arteries treated with one concentration response curve to noradrenaline in presence of losartan before angiotensin II stimulation.
 [a] $P < 0.05$ indicates statistically significant differences between rabbits fed on a control diet and rabbits fed on a diet enriched with cholesterol.
 [b] $P < 0.05$ indicates statistically significant differences with respect to control (Ang II).
 [c] $P < 0.05$ indicates statistically significant differences between arteries treated with losartan and arteries treated with one concentration response curve to noradrenaline in presence of losartan before angiotensin II stimulation.

Table 3
Maximal response and pEC$_{50}$ to noradrenaline in endothelium-intact aorta from control (CD) and hypercholesterolemic (HD) rabbit.

	E_{max} (mg)	pEC$_{50}$ (log M)	E_{max} (mg)	pEC$_{50}$ (log M)
	CD		HD	
NA	6989 ± 709	6.40 ± 0.05	7805 ± 1372	6.28 ± 0.08
NA/Ang II	5716 ± 815	4.77 ± 0.15^a	8163 ± 1258	6.55 ± 0.05^a
NA + Los	6228 ± 839	6.11 ± 0.25	7136 ± 976	5.31 ± 0.18^a
NA/Ang II + Los	7333 ± 2239	4.95 ± 0.04	7268 ± 1338	5.64 ± 0.18^b

E_{max}: maximal response; NA: noradrenaline; NA/Ang II: arteries treated with one concentration response curve to angiotensin II before stimulation with noradrenaline; NA + Los: arteries incubated with losartan (Los); NA/Ang II + Los: arteries treated with one concentration response curve to angiotensin II in presence of losartan before stimulation with noradrenaline.
 [a] $P < 0.05$ indicates statistically significant differences with respect to control (NA).
 [b] $P < 0.05$ indicates statistically significant differences between arteries treated with one concentration response curve to angiotensin II before noradrenaline stimulation and arteries incubated with losartan during concentration response curve to angiotensin II before noradrenaline stimulation.

negative 10 min after angiotensin II stimulation, although no differences were found 10 min after noradrenaline stimulation. The P_m returned to the baseline levels 20 min after washing for both the hypercholesterolemic and control angiotensin II-stimulated arteries. However, in hypercholesterolemic but not in control arteries P_m did not return to the baseline level and the membrane remained hyperpolarised after noradrenaline stimulation (Fig. 3).

4. Discussion

Rabbits fed a diet enriched with cholesterol showed higher plasma levels of cholesterol and LDL cholesterol than animals fed a control diet. No differences were observed in HDL cholesterol, triglycerides, blood glucose levels, body weight and mean arterial blood pressure between both diet groups. These results are in agreement with previous studies (Jerez et al., 2008) and support the view that the present was a non-obese, non-diabetic, hypercholesterolemic model without associated risk factors.

The present findings show endothelial dysfunction in aortic rings from hypercholesterolemic rabbits. These data are in agreement with previous studies (Verbeuren et al., 1986; Sorensen et al., 1994; Jerez et al., 2008). The endothelial dysfunction classification is validated by the impairment of the relaxation in response to acetylcholine, an endothelium dependent vasodilator, in arteries from animals with experimental hypercholesterolemia.
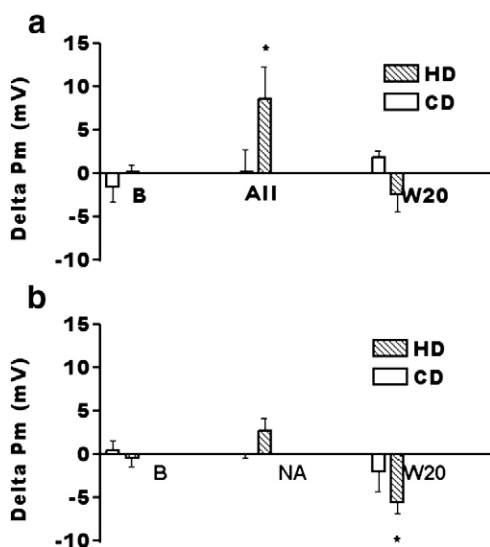
As was stated in the introduction, it is known that intact endothelium increase angiotensin II-desensitisation in the rabbit aorta (Jerez et al., 2001). There are two mechanisms involved in the development of angiotensin II-tachyphylaxis: one involves the influence of the endothelium and one occurs at the level of the smooth muscle and is considered to be endothelium-independent. The endothelium-dependent tachyphylaxis is related to the intrinsic contractile property and disappears after the 90 min washing period. The endothelium-independent tachyphylaxis is related with the loss of affinity and cannot be reversed by increasing the recovery time (Jerez et al., 2004). In the present work, endothelium-intact aortic rings from hypercholesterol-

Fig. 3. Effects of angiotensin II (a) and noradrenaline (b) on the resting membrane potentials (P_m) of arteries from rabbits fed a control (CD) or hypercholesterolemic diet (HD). Values are expressed as differences (Delta). B: P_m after 1 min of angiotensin II or noradrenaline stimulation minus P_m at baseline conditions. AII or NA: P_m after 10 min of angiotensin II or noradrenaline stimulation minus P_m after 1 min of angiotensin II or noradrenaline stimulation, respectively. W20: P_m measured 20 min after washing following angiotensin II or noradrenaline stimulation minus P_m in baseline conditions. Each bar represents the mean of 10 experiments and vertical lines indicate S.E.M. *$P<0.05$ indicates differences statistically significant with respect to baseline conditions.

emic rabbits showed a shift to the right of the second concentration response curve to angiotensin II. However, in endothelium-removed arteries, an increase of the intrinsic activity in the second exposure to angiotensin II was observed and no differences in affinity between the first and second concentration response curve were found. These results may indicate that in spite of the dysfunction observed in hypercholesterolemic conditions, the endothelium preserves its modulator role by preventing the increase in the contractile response in endothelium-intact arteries. The disappearance of endothelium independent tachyphylaxis in endothelium-removed aortic rings from hypercholesterolemic rabbits would imply changes in the regulatory property of vascular smooth muscle. These changes may be unmasked by the absence of a dysfunctional endothelium. Concordant with this result, Kuttan and Sim (1993) have stated that tachyphylaxis is associated with changes at the receptor level, i.e., changes in affinity and coupling efficiency.

In addition to homologous desensitisation to angiotensin II, endothelium-dependent cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors has been demonstrated (Jerez et al., 2004). Taking into account that hypercholesterolemia induces endothelial dysfunction, the present work evaluated the influence of a high cholesterol diet on the interaction of α_1-adrenoceptors and angiotensin AT_1 receptors at the agonist-receptor level. Desensitisation to angiotensin II in hypercholesterolemic but not in control rabbit aortic rings was found after adrenoceptor stimulation with noradrenaline. Previous reports (in physiological conditions) have shown that exposure to elevated catecholamines or other receptor-agonists in vivo or in vitro results in downregulation of angiotensin AT_1 receptors or mRNA in rat or rabbit aorta (Du et al., 1997). The disagreement with the present results may be due to the different noradrenaline incubation and recovery periods used. In the present work the aim was to study early time effects. Thus, exposure to the agonist was shorter. Blocking α_1-adrenoceptors with prazosin reduced the affinity to angiotensin II both in control and hypercholesterolemic arteries. This result could be explained, as was stated in the introduction, by angiotensin II-facilitation of neurotransmitter release from the presynaptic nerve terminals or noradrenaline-sensitisation of angiotensin II response. In this regard, it has been found that a dense adrenergic nerve supply penetrates together with the vasa vasorum into the rabbit aortic walls (Kienecker and Knoche, 1978).

An increasing number of studies have suggested that homodimers and a variety of heterodimers are the basic functional form of nearly all G protein-coupled receptors (Prinster et al., 2005). Receptor homo- or heterodimerisation leads to so-called intramembrane (or horizontal) interactions, which means that the pharmacology for agonists and/or antagonists of a given receptor usually changes (i) when it forms heteromers with another receptor and/or (ii) when the partner receptor in the heteromer is activated. This is due to conformational changes in the receptors transmitted within the receptor-receptor interface at the plane of the membrane bilayer, not excluding a G-protein-mediated cooperativity in the plane of the membrane (Franco et al., 2008). The angiotensin AT_1 receptor is also a member of the Family A of G protein-coupled receptors, which couples to G protein-dependent and -independent signalling pathways (Oro et al., 2007). Many studies have reported the formation of homo- and heterodimeric complexes of angiotensin AT_1 receptors (AbdAlla et al., 2000; Barki-Harrington et al., 2003). The functional relevance of angiotensin AT_1 hetero-oligomerisation has been reported in several systems, including its association with β2 and β1-adrenoceptors, which lead to cross-inhibition of receptor signalling following β-adrenoceptor or angiotensin AT_1 receptor antagonist treatment (Karip et al., 2007).

Taking into account these data from the literature, the present work analysed the hypothesis that a prior stimulus of α_1-adrenoceptors with noradrenaline would induce conformational changes in angiotensin AT_1 receptors and cause desensitisation to angiotensin II in hypercholesterolemic arteries. To check this hypothesis, arteries were incubated with prazosin to block α_1-adrenoceptors before exposure to noradrenaline

and to avoid interaction with angiotensin AT_1 receptors. Angiotensin II-desensitisation induced by pretreatment with noradrenaline was increased by prazosin in such condition. Likewise, to avoid possible conformational changes induced during noradrenaline-stimulation, arteries were incubated with losartan to block angiotensin AT_1 receptors. Concordant with the results obtained with prazosin, losartan did not block desensitisation to angiotensin II induced by previous stimulus with noradrenaline but was able to increase it. These results suggest that heterologous desensitisation to angiotensin II does not imply necessary physical interactions between both receptors at the plane of the membrane bilayer.

In a previous work, Jerez et al. (2008) found a depolarising effect of angiotensin II in hypercholesterolemic rabbit aortic rings. Currently, there is some evidence of a close relationship between membrane polarisation and hormone-receptor affinity (Kuttan and Sim, 1993; Jerez et al., 2001, 2004). Thus, the present work investigated the hypothesis that noradrenaline could modify the resting potential, similar to angiotensin II. Experiments showed that membrane potential from hypercholesterolemic arteries did not recover to baseline levels 20 min after washing and removal of noradrenaline from the bath. The resting potential was about 30% more negative. In contrast with the angiotensin II-effect, it has been shown that membrane potential remains hyperpolarised after exposure to noradrenaline. Therefore, this mechanism may have a role in the loss of affinity to angiotensin II induced by noradrenaline stimulation. Further studies are necessary to clarify the underlying mechanism of this phenomenon.

Desensitisation to noradrenaline response in control and sensitisation in hypercholesterolemic arteries were found after angiotensin II receptor stimulation. Data from the literature report that angiotensin II may facilitate aorta smooth muscle hypersensitivity through alfa$_{1D}$ adrenoceptors expression (Villalobos-Molina and Ibarra, 2005). However, increased α_1-adrenoceptors expression subsequent to enhanced levels of angiotensin II during the development of the hypercholesterolemia may not account for the sensitisation found in hypercholesterolemic arteries as contractile response and affinity to noradrenaline were similar in control and hypercholesterolemic arteries. Considering data from the literature previously mentioned, it could be hypothesised that the formation of angiotensin AT_1 receptors–α_1-adrenoceptors hetero-dimers occurs following angiotensin II exposure. These complexes may account for the desensitisation and sensitisation to noradrenaline observed in control and hypercholesterolemic arteries, respectively. Therefore, angiotensin AT_1 receptor stimulation may modify subsequent response to noradrenaline. In this regard, inhibition of angiotensin AT_1 receptor with losartan may avoid or diminish this phenomenon. Results obtained in the present study demonstrated that losartan was able to desensitise noradrenaline response in hypercholesterolemic but not in control arteries. Maeso et al. (1996) demonstrated that losartan reduces the phenylephrine response in aorta from spontaneously hypertensive rats. These findings support the view that angiotensin II potentiates the effect of noradrenaline through angiotensin AT_1 receptors in some pathophysiological conditions. In addition, losartan increased desensitisation and prevented sensitisation to noradrenaline induced by pretreatment with angiotensin II in control and hypercholesterolemic rabbits, respectively. Taken together, these results suggest that agonist or antagonist may induce conformational changes of angiotensin AT_1 receptor and modify noradrenaline affinity. An allosteric model for the vascular angiotensin II receptor was postulated (Timmermans et al., 1991). According to this model, two binding sites are postulated: one (R_{out}) is located at the outer side of the plasma membrane for the agonist and the antagonist, and the other (R_{in}) is located at the inner side of the membrane for the coupling factor of the receptor. By binding to R_{out}, losartan would induce conformational changes resulting in a reduction of the affinity of R_{in} for the coupling factor. Differences between control and hypercholesterolemic response would be due to membrane composition. Numerous authors reported that arteries from hypercholesterolemic animals were stiffer (Gillies and Robinson, 1988; Chen et al., 1995), suggesting that conformational changes may be different.

In summary, the current study suggests that hypercholesterolemia induces vascular changes that may alter hormone-receptor interactions. Homologous desensitisation of endothelium-independent to angiotensin II disappears and cross talk between α_1-adrenoceptors and angiotensin AT_1 receptors is modified with respect to physiological conditions.

These studies demonstrate for the first that hypercholesterolemia modifies homologous desensitisation and the interaction between α_1-adrenoceptors and angiotensin AT_1 receptors by changing vascular function in a model of hypercholesterolemia without associated hypertension. This early tissue dysfunction suggests a mechanism by which hypercholesterolemia could be involved in the onset and progression of chronic vascular diseases such as hypertension and arteriosclerosis.

Acknowledgements

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